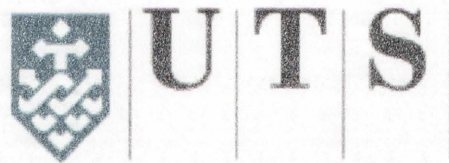


# **Role of androgens in vascular smooth muscle cell calcification**

**Tania Tsatralis**

A thesis submitted in fulfilment of the requirements for the degree of  
Master of Science



**University of Technology, Sydney**

University of Technology, Sydney  
Australia

Submitted May 2012

***"I have not failed. I've just found 10,000 ways that won't work"***

**Thomas Edison**

**1847 - 1931**

# Certificate of authorship/originality

I certify that the work of this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.



Tania Tsatralis



## Acknowledgments

My most heartfelt thanks and eternal gratitude goes to Lucinda McRobb. Thank you for your guidance. Thank you for your endless patience and constant support during what turned out to be a challenging project. I'm grateful that I had the opportunity to work with and learn from you. If I can in any way call myself a descent scientist I owe it to you.

Thank you to my supervisor Alison Heather for many years of support and for encouraging me to do a Masters degree before I even knew I wanted to do one. Thank you to David Van Reyk for all your encouraging words and for all the work you put into proof reading this thesis.

During my candidature I was fortunate to be surrounded by a group of friends, without whose help and support, I never would have made it through with my sanity intact. My biggest thank you goes to Joanne Tan for always making sure I was ok, for always providing much needed encouragement and for answering a million-and-one of my questions every day. I could never have done this without you. Thank you to Kate Shearston for emergency cake runs (read: therapy sessions) to Buzzbur Café and for helping me format this thesis, to Francesca Charlton for making me laugh when I needed it most, to Eleanore Liong for being my lab mum (and the lab clown!) and to Pat Pisansarakit for much needed company and advice during the many, many, many, many, many hours I spent in

the tissue culture lab. Thank you to all members of the Gene Regulation Group, past and present, for making my stay the HRI a memorable one.

Thank you to my mum Hrisoula and my dad Steve, who, never having had access to the educational opportunities so readily available to me, always encouraged me to educate myself and strive for the best. Of course, a new laptop from dad, dinners from mum (delivered to me at home) and “how are you coping” texts from my sister Anastasia from the other side of the globe, cannot go unmentioned.



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# Abbreviations

<b>ALP</b>	Alkaline phosphatase
<b>AP-1</b>	Activating protein-1
<b>AR</b>	Androgen receptor
<b>ART</b>	Androgen replacement therapy
<b>β-GP</b>	β-Glycerophosphate
<b>BCASMC</b>	Bovine coronary artery cells
<b>BMP</b>	Bone matrix protein
<b>BSP</b>	Bone sialoprotein
<b>Ca</b>	Calcium
<b>Cbfa1</b>	Core-binding factor alpha-1
<b>CPA</b>	Cyproterone
<b>CVC</b>	Calcifying vascular cells
<b>CVD</b>	Cardiovascular disease
<b>Dex</b>	Dexamethasone
<b>DHT</b>	5α-dihydrotestosterone
<b>E<sub>2</sub></b>	17-β-estradiol
<b>EC</b>	Endothelial cells
<b>ECM</b>	Extra-cellular matrix
<b>ERK1/2</b>	Extracellular signal-related kinase 1/2
<b>ERSD</b>	End-stage renal disease
<b>ERα</b>	Estrogen receptor-α
<b>ERβ</b>	Estrogen receptor-β
<b>FBS</b>	Foetal bovine serum
<b>GPR30</b>	G-protein coupled receptor 30
<b>HASMC</b>	Human aortic smooth muscle cells
<b>HDL</b>	High-density lipoprotein
<b>HDL-C</b>	High-density lipoprotein cholesterol
<b>HERS</b>	Heart and Estrogen/Progestin Replacement Study
<b>HF</b>	Hydroxyflutamide
<b>HRT</b>	Hormone replacement therapy
<b>Hsp</b>	Heat shock proteins
<b>IL-6</b>	Interleukin-6
<b>JNK</b>	c-Jun N-terminal kinase
<b>LDL</b>	Low-density lipoprotein
<b>LDL-C</b>	Low-density lipoprotein cholesterol
<b>MAPK</b>	Mitogen-activated protein kinase
<b>MASMC</b>	Mouse artery smooth muscle cells
<b>MGP</b>	Matrix-Gla protein
<b>MMP</b>	Matrix metalloproteinases
<b>MPP</b>	MPP dihydrochloride hydrate
<b>Msx2</b>	Msh homeobox-2
<b>NFκB</b>	Nuclear factor κB
<b>NO</b>	Nitric oxide
<b>OPG</b>	Osteoprotegerin
<b>OPN</b>	Osteopontin
<b>Osx</b>	Osterix



<b>Pi</b>	Inorganic phosphate
<b>PI3K/Akt</b>	Phosphoinositide-3 kinase
<b>PHTPP</b>	Phenyl-bis triflouromethyl pyrazole pyrimidin
<b>POCS</b>	Polycystic ovary syndrome
<b>PPI</b>	Inorganic pyrophosphate
<b>RANKL</b>	Receptor activator of nuclear $\kappa$ B ligand
<b>Runx2</b>	Runt-related transcription factor
<b>SRE</b>	Steroid response element
<b>SMC</b>	Smooth muscle cells
<b>T</b>	Testosterone
<b>TBP</b>	TATA box-binding protein
<b>(TNF)<math>\alpha</math></b>	Tumour necrosis factor- $\alpha$
<b>VSMC</b>	Vascular smooth muscle cells
<b>WHI</b>	Women's Health Initiative
<b>WHI-CACS</b>	Women's Health Initiative Coronary-Artery Calcium Study



## Abstract

Calcification is a common feature of advanced atherosclerotic lesions and is a clinically significant predictor of cardiovascular events. Coronary calcification is more prevalent in men than age-matched women. However, atherosclerotic calcification increases in postmenopausal women, who present with lower levels of estrogen, suggesting that sex hormones play a critical role in its pathogenesis and progression. This has implications for hormone therapy treatment that is used to treat age-related conditions such as osteoporosis and menopause. Extensive observational studies into estrogen replacement therapy have revealed that postmenopausal women treated with estrogen exhibit less extensive atherosclerotic calcification. The effects of androgens on atherosclerotic calcification have, however, received little attention and consequently its mechanisms remain poorly understood. This study therefore explored the effects of androgens on atherosclerotic calcification.

*In vitro* studies postulate vascular smooth muscle cell (VSMC) differentiation into mineralising osteoblast-like cells as a key mediator of atherosclerotic calcification. Given the gender disparity in atherosclerotic calcification we hypothesised that androgens promote differentiation of VSMC into mineralising osteoblast-like cells. Therefore, the aims of this study were to 1) examine the effects of androgens in vascular smooth muscle cell differentiation and calcification and 2) elucidate the molecular mechanisms of androgen action in this process, using phosphate-induced bovine and murine *in vitro* models of calcification.

This study demonstrated that co-treatment of bovine coronary artery smooth muscle cells (BCASMC) with phosphate and testosterone (T) and dihydrotestosterone (DHT) promoted calcification. Investigation of the molecular mechanisms underlying calcification in the bovine model revealed T-stimulated calcification was estrogen receptor (ER) driven. DHT, however, mediated its effects via the androgen receptor (AR). Further investigation of molecular mechanisms showed DHT regulated ALP activity whereas T did not. T,

therefore, promoted calcification in an ER-driven, ALP independent pathway in contrast to DHT, which mediated its effects via an AR-driven, ALP dependent pathway.

A primary mouse cell-based calcification model was also established. In contrast to the bovine model, it was found that T and DHT treatment did not promote calcification in the murine model. The lack of androgen promotion of calcification in this model was associated with the absence of ALP activity. The conclusion drawn from the bovine model, of a mechanistic role for ALP in the DHT/AR driven mineralisation but not for T-driven mineralisation, suggested that in the murine cells an ER pathway is not functioning.

In conclusion, the studies presented in this thesis demonstrate that T and DHT promote differentiation of vascular smooth muscle cells into osteoblast-like cells capable of mineralisation. T and DHT mediate calcification via alternative pathways that can involve AR and ERs. A potential mechanistic role for ALP in DHT/AR-driven mineralisation has been established.